United States Patent **5,362,628**
Haugland , et al. **November 8, 1994**

Fluorescent haloalkyl derivatives of reporter molecules well retained in cells

Abstract

The subject invention provides a method for analyzing the metabolic activity in cells by improving the retention of a detectable reporter molecule only in intact cells where a particular enzyme is present. In particular, improved retention results from a two part process involving conjugation of haloalkyl-substituted derivatives of a ***reporter molecule*** with intracellular cysteine-containing peptides while unblocking the ***reporter molecule***. The method for analyzing metabolic activity of cells involves the use of a substrate having the form XR-REPORTER-BLOCK wherein -BLOCK is a group selected to be removable by action of a specific analyte, to give REPORTER spectral properties different from those of the substrate, ***-REPORTER- is a molecule*** that, when no longer bound to BLOCK by a BLOCK-REPORTER bond, has spectral properities different from those of the substrate, and XR-- is a haloalkyl moiety that can covalently react with an intracellular thiol (Z--S--H) to form a thioether conjugate (Z--S--R--). After the substrate enters the cells, the analyte removes BLOCK to make REPORTER detectable by the change in spectral properties, and the haloalkyl XR reacts with the intracellular thiol to form the thioether conjugate inside the cells, which is well-retained in the cells.

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Appl. No.: **026633**

Filed: **March 5, 1993**

Current U.S. Class: **435/18**; 435/4; 435/14; 435/19; 435/20; 435/21; 435/22; 435/23; 435/24; 435/25; 435/26; 435/29; 436/63; 436/66; 530/300; 530/802; 536/4.1; 536/18.1

Intern'l Class: C12Q 001/00; C12Q 001/34; G01N 033/40; A61K 037/02; C07H 015/24

Field of Search: 435/18-26.14.29.4 436/63.66 530/300.802 536/18.1,4.1